

Nonlinear grassland responses to past and future atmospheric CO₂

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Carbon sequestration in soil organic matter may moderate increases in atmospheric CO₂ concentrations (C_a) as C_a increases to more than 500 μmol mol⁻¹ this century from interglacial levels of less than 200 μmol mol⁻¹ (refs 1–6). However, such carbon storage depends on feedbacks between plant responses to C_a and nutrient availability^{7,8}. Here we present evidence that soil carbon storage and nitrogen cycling in a grassland ecosystem are much more responsive to increases in past C_a than to those forecast for the coming century. Along a continuous gradient of 200 to 550 μmol mol⁻¹ (refs 9, 10), increased C_a promoted higher photosynthetic rates and altered plant tissue chemistry. Soil carbon was lost at subambient C_a, but was unchanged at elevated C_a where losses of old soil carbon offset increases in new carbon. Along the experimental gradient in C_a there was a nonlinear, threefold decrease in nitrogen availability. The differences in sensitivity of carbon storage to historical and future C_a and increased nutrient limitation suggest that the passive sequestration of carbon in soils may have been important historically, but the ability of soils to continue as sinks is limited.

The concentration of CO₂ in the atmosphere has increased dramatically since the Last Glacial Maximum, most recently owing to fossil fuel burning and land conversion to agriculture.

This increase in C_a has focused attention on the role of terrestrial ecosystems in sequestering anthropogenic CO₂ (refs 2, 5, 7, 11, 12). The long-term consequences of rising C_a on C sequestration are highly dependent on feedbacks between plant responses to C_a and nutrient dynamics^{7,8,13}. Plant growth is often enhanced with increases in C_a (refs 6, 14), sometimes leading to changes in plant tissue chemistry and organic inputs to soils^{15,16}. These and other feedbacks controlled by microbial processes may either increase^{13,17} or decrease^{7,8,18} nutrient availability, and mediate the long-term ability of ecosystems to sequester C^{7,8,19}. For C sequestration to be important at decadal and century timescales, nutrient availability must not hinder higher plant production and new organic C must be stabilized in soil pools with relatively long turnover times. The partitioning of C among soil organic matter (SOM) pools with different turnover rates is thus a crucial determinant of C sequestration in many systems and is tightly coupled with plant tissue chemistry and nutrient dynamics^{13,16,18}.

A field experiment⁹ in an intact C₃/C₄ grassland in central Texas provided a continuous gradient of C_a from 200 to 550 μmol mol⁻¹ permitting the measurement of critical threshold and nonlinear responses to past, present and future atmospheric CO₂. Plant and ecosystem properties, including water-use efficiency, photosynthesis, respiration rates and primary productivity, often change with rising C_a, but it is not likely that all such responses were or will be linear^{3,20,21}. Physiological thresholds²⁰, transient or acclimatory responses²², and the strong coupling of plant and soil responses¹⁸ are examples of mechanisms that may drive nonlinear processes in nature²³. Nonlinear and threshold responses are the focus of several new international programmes²³ and may explain some of the apparent contradictory results observed in recent CO₂ studies^{8,15}. Furthermore, research on how intact ecosystems respond to both past and future C_a provides a context that can demonstrate the sensitivity of C dynamics to changes that have already occurred as well as those forecast for the coming century. Extrapolation from experiments that impose step changes in C_a is complicated by the possibility that plants may evolve as C_a changes more slowly in nature. There is some evidence, however, that perennial plants have not evolved quickly enough to be closely adapted to current C_a (ref. 24).

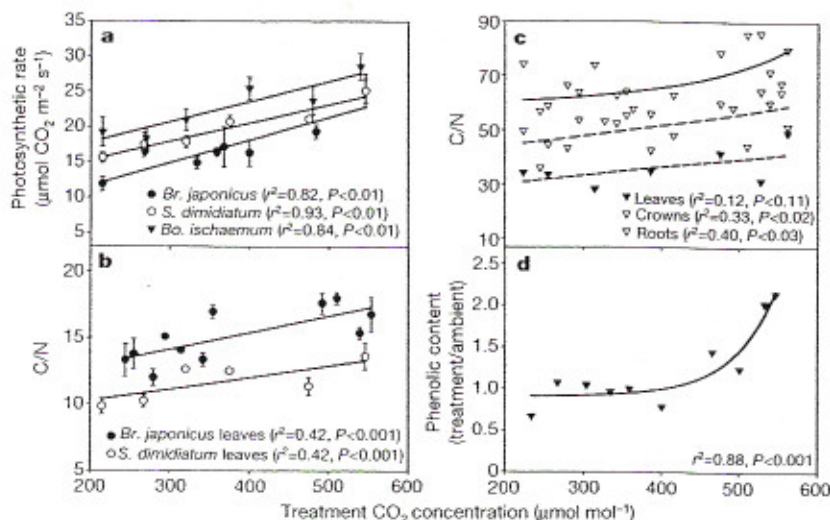


Figure 1 Effects of CO₂ treatment on various species. **a**, Maximum CO₂ assimilation for three species (*Bothriochloa ischaemum*, *Solanum dimidiatum*, *Bromus japonicus*) in 1999, showing a significant positive relationship between maximum CO₂ assimilation and treatment CO₂ in all species ($P < 0.01$). **b**, C/N ratio for leaves from the two C₃ species show a positive, linear increase for both species with increasing treatment CO₂. **c**, C/N ratio for *Bo. ischaemum* roots, crowns and leaves. Roots showed an exponential increase

in C/N ratio with increasing CO₂ ($P < 0.03$); crowns showed a positive, linear increase ($P < 0.05$). **d**, Relative change in phenolic concentrations in *Bo. ischaemum* roots (expressed relative to ambient values). There was a strong, exponential increase in root phenolic content ($P < 0.001$), with an apparent threshold at C_a slightly above ambient levels.

Table 1 Calculated carbon production and storage

	Year	Elevated (550–350 $\mu\text{mol mol}^{-1}$)	Subambient (365–200 $\mu\text{mol mol}^{-1}$)	R^2 (P value)	Superambient/ subambient	Pretreatment superambient/subambient
Aboveground net primary production ($\text{g m}^{-2} \text{yr}^{-1}$)	1996–2000	1,047.5 (64.9)	683.9 (52.2)	0.35 (0.008)	1.52	0.83
Belowground net primary production 0–30 cm ($\text{g m}^{-2} \text{yr}^{-1}$)	1996–1999	294 (24.6)	185 (22.8)	0.16 (0.09)	1.59	1.03
Soil CO_2 flux ($\mu\text{g m}^{-2} \text{sec}^{-1}$)	1996–2000	4.02 (0.13)	2.85 (0.17)	0.46 (0.001)	1.41	1.13
Root biomass 0–30 cm (g m^{-2})	1998	173.0 (39.4)	102.0 (16.2)	0.25 (0.02)	1.69	0.84
Soil organic carbon 0–15 cm (g m^{-2})	1996–2000	4,442 (175)	3,656 (120)	0.32 (0.05)	1.22	1.05

R^2 and P values are from best-fit regressions of variables on C_a over subambient through elevated concentrations. s.e.m., the standard error of the mean, shown in parentheses after the mean value. For all data other than root biomass, s.e.m. is determined as the standard error for annual means; for root biomass it is the standard error between section means ($n = 10$).

Along the experimental gradient, plants responded to higher C_a by increasing photosynthesis and net primary production (Fig. 1a, Table 1). As treatment CO_2 increased, maximum CO_2 assimilation rates increased linearly for both C_3 and C_4 plants¹⁰ (Fig. 1a; $P < 0.01$). Associated with this increase in CO_2 assimilation was a 50% increase in above- and belowground net primary production at elevated CO_2 compared to subambient CO_2 (Table 1). Tissue chemistry was altered as well, with an increase in tissue C/N with higher C_a and an exponential increase in phenolic concentration (Fig. 1b–d). C_a and species type were highly significant predictors of C/N, with C/N positively correlated with C_a (analysis of covariance (ANCOVA): $P < 0.001$ for C_a ; $P < 0.001$ for species). The concentration of phenolic compounds in roots of one of the dominant species in the system, the C_4 grass *Bothriochloa ischaemum*, showed a strong threshold effect, with little variation in plants grown at subambient C_a , but an exponential increase above ambient CO_2 (Fig. 1d, $P < 0.001$).

Soil C storage and belowground metabolism were greatly altered. Despite a linear increase in photosynthesis along the gradient, soil C storage was much more sensitive to subambient than to elevated C_a (Fig. 2a). At subambient C_a , bulk soil C stocks decreased by 11%, or 450 g m^{-2} , between 1996 and 2000 (Table 2). However, there was no concomitant increase in soil C storage at elevated C_a (Fig. 2a), with soil C increasing by a modest 3.3% (144 g m^{-2}) over the same time period (Table 2). The relationship between treatment CO_2 and the change in bulk soil organic C over three years follows an asymptotic function (Fig. 2a, $P < 0.05$), suggesting that the ability of soils to act as sinks for anthropogenic CO_2 will slow or reach saturation.

Accompanying altered soil C storage was an important change in soil organic matter chemistry. Total organic matter C/N was linearly associated with treatment C_a (Fig. 2c, $P < 0.01$), in a pattern similar to that observed for plant tissue chemistry. There was also a divergence in patterns of soil respiration at super- versus subambient C_a . Soil CO_2 flux at peak plant growth was 40% higher at elevated than at subambient C_a , suggesting that much of the increase in C fixed with rising C_a is lost to microbial or root respiration⁵ (Table 1).

The changes observed in particulate organic matter (POM) demonstrate a shift in the balance between new and old SOM. POM is a relatively labile class of SOM, with a residence time of between 10 and 50 years^{11,25,26}. The 14% loss in POM carbon at subambient C_a parallels the loss in total organic C (Table 2). However, in contrast to total organic C, POM C increased linearly with treatment CO_2 , even at elevated C_a (Fig. 2b). These findings indicate that at elevated C_a , increases in POM C were largely offset by losses in the older, mineral-associated organic matter²⁷ (Table 2). Even within the POM class, there were increases at elevated C_a in the two most labile fractions (free and macroaggregate POM), while there was a decrease in the most recalcitrant fraction (microaggregate POM)²⁶ (Table 2). This represents a change in ecosystem C partitioning to faster cycling organic matter^{11,16,26}, which may explain why higher C assimilation and production did not lead to increased C sequestration. Our result is similar to those of other studies that reported that at low nutrient availability and elevated CO_2 , carbon was lost from the mineral-bound fraction of SOM²⁵. Similarly, an annual grassland exposed to a doubling of C_a had

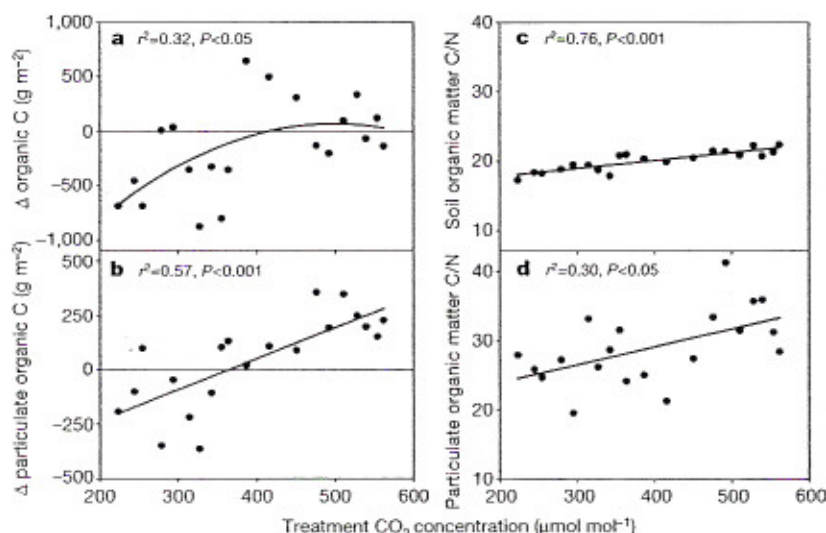


Figure 2 Effect of CO_2 treatment on soil carbon storage. **a**, Change in organic C stocks (0–15 cm) between 1997 and 2000. Values are the difference between section means in 1997 and 2000 determined using four subsamples per 5-m section per year. There was a quadratic relationship between the change in soil C stocks and treatment CO_2 ($P < 0.05$). The linear fit for these data was not significant. **b**, Significant, linear change in particulate

organic matter (POM) carbon between 1997 and 2000 ($P < 0.001$). **c**, There was a significant, linear increase in bulk soil organic matter (SOM) C/N with treatment CO_2 in December 2000 ($P < 0.001$). **d**, POM C/N for samples collected in December 2000 ($P < 0.05$). Values are the means of four subsamples per section.

Table 2 Pools and changes in soil organic carbon and particulate organic carbon

	Treatment leg	December 2000 0–15 cm (g m ⁻²)	Change (g m ⁻²) 1997–2000	Relative change (%)
Total soil organic matter C	Superambient	4,442 (175)	144 (92)	3.3
	Subambient	3,656 (120)	-450 (100)	-11
Particulate organic matter C—Free	Superambient	186.1 (23.7)	86.5 (22.0)	70
	Subambient	158.2 (16.8)	-26.9 (6.3)	-16
Macroaggregate	Superambient	723.4 (38.7)	193.3 (33.9)	36
	Subambient	626.6 (39.8)	-104.0 (56.8)	-14
Microaggregates	Superambient	87.5 (6.7)	-21.6 (22.6)	-24
	Subambient	65.9 (6.4)	-5.3 (5.5)	-9
Total particulate organic matter C	Superambient	975.4 (33.9)	258.2 (70.1)	35
	Subambient	857.3 (56.8)	-132.3 (68.2)	-14
Mineral-associated organic matter C	Superambient	3,719 (195)	-123 (96)	-3.3
	Subambient	3,030 (118)	-346 (121)	-9.8

s.e.m., the standard error of the section means ($n = 10$), shown in parentheses after the mean value.

higher ecosystem C uptake and belowground allocation but little extra C storage⁵. Much of the increased C was partitioned to rapidly cycling pools that make a negligible contribution to long-term storage because of their small size and relatively high turnover rates.

The feedback between plant responses to C_a and nutrient dynamics is vital in determining C sequestration in ecosystems^{7,8,18}. Nitrogen mineralization rates decreased dramatically and non-linearly with increasing CO_2 ($P < 0.01$), with the largest changes occurring at subambient concentrations (Fig. 3). Net N mineralization was three times higher at 200–240 $\mu\text{mol mol}^{-1}$ CO_2 than at 530–550 $\mu\text{mol mol}^{-1}$. Because of the changes in the chemical composition of detritus and increased C supply, microbes at high CO_2 may need to mineralize older, mineral-associated SOM to meet their nutritional requirements. As a result, there was a decrease in plant-available N as a consequence of microbial immobilization and a loss in C stored in mineral-associated fractions of organic matter. Some workers have concluded that suppressed N availability under elevated CO_2 may increase C storage by suppressing decomposition rates^{8,18}, but we found that there were only modest gains in soil C storage at the lowest N availability. In contrast to other grassland

CO_2 studies^{6,14,21}, our results are apparently a consequence of altered plant litter chemistry rather than an indirect effect of altered soil water status, as increases in plant water-use efficiency along the gradient¹⁰ were offset by higher plant biomass (data not shown). Increases in C_a resulted in higher nitrogen-use efficiency by plants¹⁰, but a threefold decrease in nitrogen availability will probably have a detrimental effect on long-term plant productivity and, ultimately, on ecosystem carbon storage.

Higher net primary productivity^{5,7}, altered plant tissue chemistry²⁷, modifications of SOM composition and stocks^{5,11,25}, and changes in nutrient availability^{13,18} with increases in C_a suggest that both forests and grasslands are sensitive to rising CO_2 . The capacity of future ecosystems to act as sinks for anthropogenic CO_2 will be determined by feedbacks among ecosystem processes^{7,18} and will be sensitive to the location of specific thresholds that influence the magnitude of the change in ecosystem dynamics²³. In this grassland, soil C stocks and net N mineralization are much more sensitive to subambient than elevated C_a , indicating that we are currently at an important threshold. Soils may have played a role in passively sequestering C since the last interglacial period, but their ability to continue to act as a C sink may be limited by nutrient availability. To assess the impacts of rising CO_2 on carbon sequestration patterns and nutrient dynamics requires knowledge of potential threshold responses and the legacy of historical and prehistorical changes. □

Methods

Experimental system

Two parallel, elongated chambers (1 m tall \times 1 m wide \times 60 m long) were constructed on a grassland dominated by the C_4 perennial grass *Bothriochloa ischaemum* (L.) Keng and Ambient air plus the C_3 perennial forbs *Solanum dimidiatum* Raf. and *Ratibida columnaris* (Sims) D. Don. pure CO_2 was injected into the eastern chamber to initiate the elevated gradient (550–350 $\mu\text{mol mol}^{-1}$), while ambient air was injected into the western chamber, initiating the subambient CO_2 gradient (365–200 $\mu\text{mol mol}^{-1}$). Gradients have been maintained during the growing season since May 1997 by altering flow rate through the chambers. At night, air flow in the chambers is reversed, maintaining a C_a gradient at 150 $\mu\text{mol mol}^{-1}$ above daytime concentrations. The chambers are divided into 5-m sections, and air is cooled and dehumidified in each section to maintain air temperature and vapour pressure deficit near ambient conditions. Our results span pre-treatment data (1996–1997) and the three complete growing seasons during which the grassland was exposed to a C_a gradient (1998–2000).

Soil analyses

Soil respiration was evaluated monthly using a LI-COR 6200. Total inorganic and organic soil carbon was determined using a two-temperature combustion procedure designed specifically for calcareous Blackland Prairie soils²⁸. Four soil cores were collected from each of the 20 sections in stratified, random positions. Total C and N were measured using a CE Instruments NC 2100 elemental analyser (ThermoQuest Italia). We measured POM in two aggregate size classes (macroaggregates ($>250 \mu\text{m}$); microaggregates (250–53 μm)) using the method described in ref. 26 to determine POM C. Mineral-associated C was determined by difference between total C and POM C. We determined POM C using four soil samples from each section ($n = 80$) that were collected in September 1997 and December 2000. We used a month-long, *in situ* open-core incubation method described in ref. 29 to measure net nitrogen mineralization.

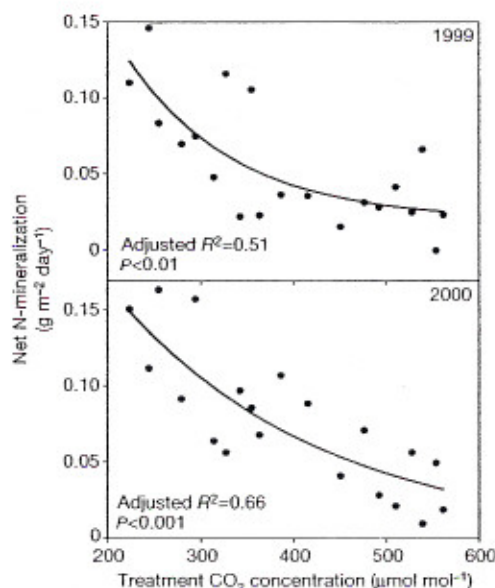


Figure 3 Net N-mineralization during the 1999 and 2000 growing seasons. There was a significant, negative exponential relationship between net N mineralization and treatment CO_2 during midsummer in both years ($P < 0.001$). During spring and autumn there were no significant differences in N mineralization rates for the subambient and elevated chambers.

Statistical considerations

The experimental system is constructed to resolve the shape of ecosystem responses to a gradient in CO_2 . The experimental design uses a regression approach to test for significant CO_2 effects based on changes in slope along the gradient. We used regression to test for a significant relationship between C_3 and the response variable using the regression wizard in SigmaPlot 5.0 for Windows (SPSS Inc.). We tested linear, logarithmic, power and hyperbolic functions to fit the data, and selected the model with the highest adjusted R^2 after examining the residual plots for normality and homoscedasticity. When models were nearly the same in their explanatory value (R^2 values within 0.05), we report results for the linear model. Because we had only a single experimental system oriented in one direction across the landscape, it is possible that the measured responses may have been influenced by some unquantified factor covarying with CO_2 treatment. However, extensive pretreatment data, including such ecosystem characteristics as soil C stocks, net primary productivity and soil respiration, revealed no such trends before fumigation (Table 1 and additional data not shown). Furthermore, the system design ensured that key environmental variables (photosynthetically active radiation, T, relative humidity, and so on) remained similar across the gradient. The absence of strong threshold responses at the transition between the two chambers provides further evidence that neither landscape position nor position within the chamber significantly influenced observations. To control for any pre-existing variation in soil organic matter, we evaluate the change in soil C stocks between 1997 and 2000 rather than absolute levels (Table 2).

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Competing interests statement

The authors declare that they have no competing financial interests.

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